PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:

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Date of mailing *(day/month/year)*	
21 July 2005 (21.07.2005)	
Applicant's or agent's file reference	**IMPORTANT NOTICE**
399	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB2003/006197	24 December 2003 (24.12.2003)	

Applicant

COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH et al

1. Notice is hereby given that the International Bureau has **communicated**, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this notice:

   AU, AZ, BY, CH, CN, CO, DZ, EP, HU, KG, KP, KR, MD, MK, MZ, RU, SY, TM, US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

   AE, AG, AL, AM, AP, AT, BA, BB, BG, BR, BZ, CA, CR, CU, CZ, DE, DK, DM, EA, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, ID, IL, IN, IS, JP, KE, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MG, MN, MW, MX, NI, NO, NZ, OA, OM, PG, PH, PL, PT, RO, SC, SD, SE, SG, SK, SL, TJ, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-*bis*)).

3. Enclosed with this notice is a copy of the international application as published by the International Bureau on 21 July 2005 (21.07.2005) under No. WO 2005/065470

4. **TIME LIMITS for filing a demand for international preliminary examination and for entry into the national phase**

   The applicable time limit for entering the national phase will, **subject to what is said in the following paragraph,** be **30 MONTHS** from the priority date, not only in respect of any elected Office if a demand for international preliminary examination is filed before the expiration of **19 months** from the priority date, but also in respect of any designated Office, in the absence of filing of such demand, where Article 22(1) as modified with effect from 1 April 2002 applies in respect of that designated Office. For further details, see *PCT Gazette* No. 44/2001 of 1 November 2001, pages 19926, 19932 and 19934, as well as the *PCT Newsletter*, October and November 2001 and February 2002 issues.

   In practice, **time limits other than the 30-month time limit** will continue to apply, for various periods of time, in respect of certain designated or elected Offices. For **regular updates on the applicable time limits** (20, 21, 30 or 31 months, or other time limit), Office by Office, refer to the *PCT Gazette*, the *PCT Newsletter* and the *PCT Applicant's Guide*, Volume II, National Chapters, all available from WIPO's Internet site, at http://www.wipo.int/pct/en/index.html.

   For filing a **demand for international preliminary examination**, see the *PCT Applicant's Guide*, Volume I/A, Chapter IX. Only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination (at present, all PCT Contracting States are bound by Chapter II).

   It is the applicant's **sole responsibility** to monitor all these time limits.

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(b) filtering and concentrating the solvent obtained in step (a) to obtain a concentrate and to recover upto 90% of the solvent;

(c) drying the concentrate obtained in step (b) in a vacuum oven at 40-50°C under vacuum at 10-25 mm of mercury to obtain the antibacterial bioactive fraction.

In an embodiment of the present invention, the organic solvent used is hexane.

In another embodiment of the present invention, the yield of hexane extract is about 1.5 to 3.0%.

In yet another embodiment of the present invention, the filtration is carried out by conventional methods.

In still another embodiment of the present invention, the concentration temperature is of 55 – 60°C.

In a further embodiment of the present invention, the antibacterial bioactive fraction thus obtained has antibacterial activity against gram positive and gram negative bacterial in the range of 200-500 ppm.

Accordingly, the present invention provides a process for the preparation of antibacterial fraction, which comprises,

i) Powdering the fruits of *Cinnamomum zeylanicum* to get a particle size 60-80 mesh.

ii) extracting of the above said material with hexane in a Soxhlet extractor at a temperature of 55-60 °C for a period of 6-8 h.

iii) filtering the above extract using Whatman filter paper no.1 to obtain the particle free extract.

iv) distilling the above extract to recover / recycle the solvent up to 90%.

v) concentrating the above particle free extract at a temperature of 55 - 60 °C

vi) drying the above concentrated extract using vacuum oven at 40-50 °C under vacuum at 10-25 mm of mercury.

vii) the product thus obtained had antibacterial activity against different Gram positive and Gram negative bacteria in the range of 200-500 ppm.

In an embodiment of the present invention, the yield of hexane extract was found to be 1.5 -3.0%.

The preparation of antibacterial fraction from the unconventional parts of *Cinnamomum zeylanicum* was done according to the flow diagram shown in Figure 1.

The novelty of the process includes:

1. This is the first report of preparation of antibacterial fraction from the unconventional parts of *Cinnamomum zeylanicum.*
2. The invention is a single step process to obtain the bioactive fraction from the unconventional parts of *Cinnamomum zeylanicum.*

The following examples are given by way of illustration of the present invention and therefore should not be constructed to limit the scope of the present invention.

Example 1

50 g fruits of *Cinnamomum zeylanicum* were powdered using mixer grinder to get a 60 mesh size. The powder was extracted using 200 ml of hexane at 60 °C for 8 h in a Soxhlet extractor. The hexane extract was filtered using Whatman filter paper No.1 and it was concentrated to recover the 150 ml of solvent. The concentrate was dried in a vacuum oven at 40 °C under 10 mm of vacuum. The yield of extract was 1.4 g.

The antibacterial assay for the extract of *Cinnamomum zeylanicum* was tested by pour plate method against *Bacillus cereus* by the method of Negi *et al.* (J. Agricultural and Food Chemistry 47, 4297-4300, 1999). To flasks containing 20 ml melted nutrient agar, different concentration of test material in propylene glycol were added. Equivalent amounts of propylene glycol were used as controls. One hundred μl (about 10^3 cfu/ml) of culture was inoculated into the flasks under aseptic conditions. The media was then poured into sterilized petri plates in quadruplet and incubated at 37 °C for 20-24 h for growth. The minimum inhibitory concentration (MIC) was reported as the lowest concentration of the compound capable of inhibiting the complete growth of the bacterium being tested. The MIC value of *Cinnamomum zeylanicum* fruit extract against *Bacillus cereus* was 250 ppm.

Example -2

The dried fruits (100 g) of *Cinnamomum zeylanicum* were powdered in a mixer grinder to get 80 mesh size. The powder was extracted with 400 ml of hexane by using Soxhlet extractor at 55 °C for 8 h. The extract was filtered using Whatman filter paper No 1. and concentrated under vacuum to recover the 360 ml of solvent. The concentrate dried at a temperature of 35 °C and under a reduced pressure at 25 mm of mercury. The yield of hexane extract was 3.0g.

The antibacterial assay for the extract of *Cinnamomum zeylanicum* was done by known

method. The MIC value of *Cinnamomum zeylanicum* fruit extract against *Bacillus subtilis* was 300 ppm.

Example -3

The dried fruits (150 g) of *Cinnamomum zeylanicum* were powdered in a mixer grinder to get 80 mesh size. The powder was extracted with 600 ml of hexane by using Soxhlet extractor at 55 °C for 8 h. The extract was filtered using Whatman filter paper No 1 and concentrated under vacuum to recover the 520 ml of solvent. The concentrate dried at a temperature of 35 °C and under a reduced pressure at 25 mm of mercury. The yield of hexane extract was 4.7g.

The antibacterial assay for the extract of *Cinnamomum zeylanicum* was done by known method. The MIC value of *Cinnamomum zeylanicum* extract against *Bacillus coagulans* was 300 ppm.

Example 4

The antibacterial assay for the extract of *Cinnamomum zeylanicum* was done by known method. The MIC value of *Cinnamomum zeylanicum* extract against *Pseudomonas aeruginosa* was 200 ppm.

Example 5

The antibacterial assay for the extract of *Cinnamomum zeylanicum* was done by known method. The MIC value of *Cinnamomum zeylanicum* extract against *Staphylococcus aureus* was 500 ppm.

The advantages of the process are:

1. The process is simple and the solvents used in this process can be regenerated for further use.
2. The raw material has no commercial value at present.

We claim:

1. A composition comprising a bioactive fraction obtained from fruits of *Cinnamomum zeylanicum* having

Moisture:	4-6%
Color:	Greenish white
Flavor:	Mild salty flavor

optionally along with one or more pharmaceutically acceptable additives.

2 A composition as claimed in claim 1, wherein the bioactive fraction is a hexane extract obtained from the fruits of *Cinnamomum zeylanicum*.

3 A composition as claimed in claim 1, wherein the composition has antibacterial activity against gram positive and gram negative bacterial in the range of 200-500 ppm.

4 A composition as claimed in claim 1, wherein the composition has antibacterial activity against *Bacillus cereus*, *Bacillus subtilis*, *Bacillus coagulans*, *Pseucomonas aeruginosa*, *Staphylococcus aureus*.

5 Use of a bioactive fraction obtained from fruits of *Cinnamomum zeylanicum* having

Moisture:	4-6%
Color:	Greenish white
Flavor:	Mild salty flavor

as an antibacterial agent.

6. Use as claimed in claim 5, wherein the bioactive fraction is a hexane extract obtained from the fruits of *Cinnamomum zeylanicum*.

7. Use as claimed in claim 5, wherein the bioactive fraction has antibacterial activity against gram positive and gram negative bacterial in the range of 200-500 ppm.

8. Use as claimed in claim 5, wherein the bioactive has antibacterial activity against *Bacillus cereus*, *Bacillus subtilis*, *Bacillus coagulans*, *Pseucomonas aeruginosa*, *Staphylococcus aureus*.

9. A process for preparing antibacterial bioactive fraction having

Moisture:	4-6%
Color:	Greenish white
Flavor:	Mild salty flavor

from the unconventional parts of *Cinnamomum zeylanicum*, said process comprising the steps of :

(a) extracting the powdered fruits of Cinnamomum zeylanicum with an organic solvent at a temperature in the range of 55-60°C for a time period in the range of 6-8 mesh.

(b) filtering and concentrating the solvent obtained in step (a) to obtain a concentrate and to recover upto 90% of the solvent;

(c) drying the concentrate obtained in step (b) in a vacuum oven at 40-50°C under vacuum at 10-25 mm of mercury to obtain the antibacterial bioactive fraction.

10. A process as claimed in claim 1 wherein the organic solvent used is hexane.

11. A process as claimed in claim 2 wherein the yield of hexane extract is about 1.5 to 3.0%.

12. A process as claimed in claim 1 wherein the filtration is carried out by conventional methods.

13. A process as claimed in claim 1 wherein the concentration temperature is of 55 – 60°C.

14. A process as claimed in claim 1 wherein the antibacterial bioactive fraction thus obtained has antibacterial activity against gram positive and gram negative bacterial in the range of 200-500 ppm.

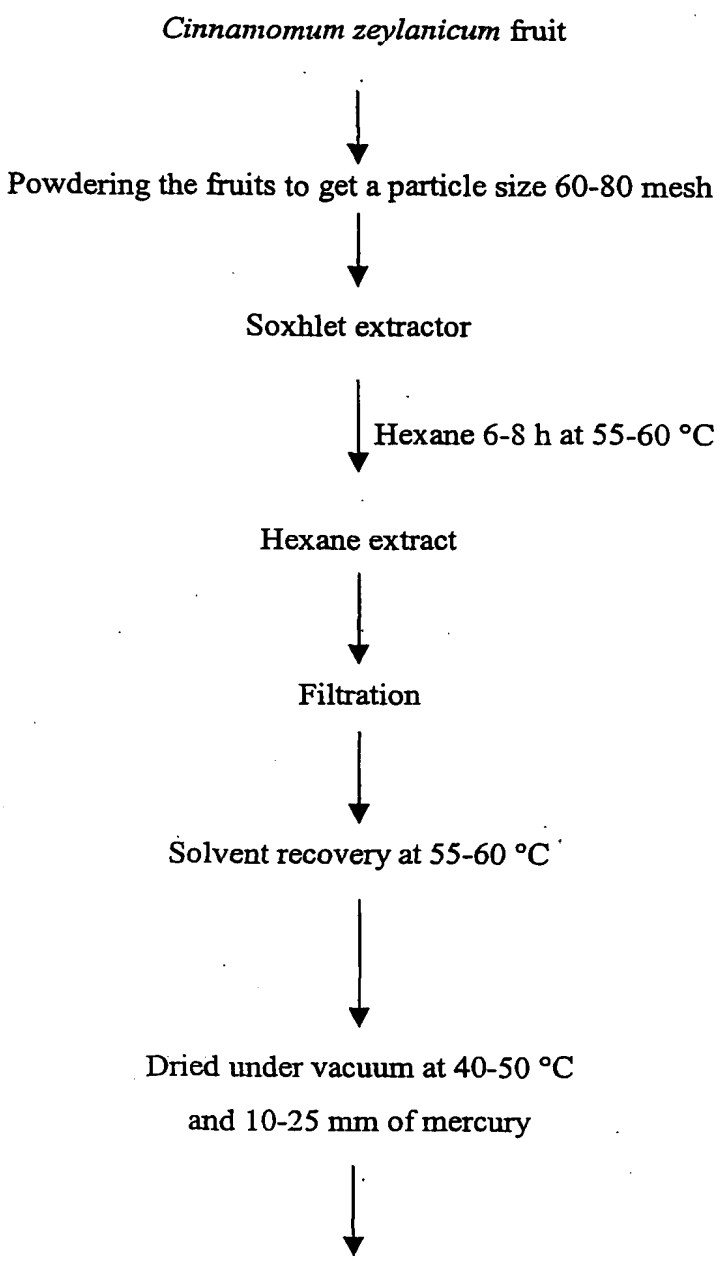
Cinnamomum zeylanicum fruit
Powdering the fruits to get a particle size 60-80 mesh
Soxhlet extractor
Hexane 6-8 h at 55-60 °C
Hexane extract
Filtration
Solvent recovery at 55-60 °C
Dried under vacuum at 40-50 °C
and 10-25 mm of mercury
Yield of antibacterial fraction (1.5-3.0%)

Figure 1

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 03/06197

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L3/3472 A61K35/78

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PATENT ABSTRACTS OF JAPAN vol. 00, & JP 55 102380 A (KUREHA CHEM. IND. CO. LTD.), 5 August 1980 (1980-08-05) abstract	1-14
A	SMITH-PALMER A. ET AL.: "Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens" LETTERS IN APPLIED MICROBIOL., vol. 26, 1998, pages 118-122, XP002288144 see abstract and tables 1-3	1-14

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 July 2004	26/07/2004

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 03/06197

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	UNGSURUNGSIE M.: "Mutagenicity of extracts from Ceylon cinnamon in the rec assay." FD. CHEM. TOXIC., vol. 22, no. 2, 1984, pages 109-112, XP002288145 see abstract and table 1	1-14
A	VALERO M. ET AL.: "Antibacterial activity of 11 essetial oils against Bacillus cereus in tyndallized carrot broth." INT. J. FOOD MICROBIOL., vol. 85, 2003, pages 73-81, XP002288146 see abstract and tables 3-5	1-14
A	HILI P. ET AL.: "Antimicrobial action of essential oils : the effect of dimethylsulphoxide on the activity of cinnamon oil." LETTERS IN APPLIED MICROBIOLOGY, vol. 24, 1997, pages 269-275, XP002288147 see abstract and tables 1-3	1-14

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 03/06197

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